PATENT
4614-0110P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	**STEEN, KLYSNER et al..**	**Conf.: 2471**
Appl. No.:	**09/620,586**	**Group: 1644**
Filed:	**July 20, 2000**	**Examiner: Belyavskyi, M.**
For:	**METHOD FOR DOWN-REGULATING GDF-8 ACTIVITY**	

DECLARATION SUBMITTED UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, DC 20231

March 17, 2004

Sir:

I, Steen Klysner, of Pharmexa A/S, Kogle Allé 6, DK-2970 Hørsholm, Denmark do hereby declare the following:

I have attached a copy of my curriculum vitae to this Declaration.

I hold a PhD in protein chemistry, I am project manager at Pharmexa A/S and I am one of the inventors of the above referenced patent application. I am familiar with the development, use and properties of the modified GDF-8 polypeptides used in the method of down-regulation described therein.

I have read and understood the subject matter of the Office Action of October 21, 2003.

The following comments are offered in support of the patentability of the instant invention.

The instant application describes a method of down-regulating growth differentiation factor 8 (GDF-8) activity in an animal by administering an immunologically effective amount of a GDF-8 polypeptide (myostatin) that has been modified by inserting one or more foreign T helper epitopes into defined portions of the GDF-8 polypeptide. By vaccinating animals with the modified GDF-8 constructs, it is possible to down-regulate the GDF -8 activity in the animal and thus effect an increase in the muscle mass of the animal.

The Examiner has cited to the disclosure on page 16, lines 24-30 of the Specification and the Barker et al. reference (US. Patent No. 6,369,201) and argues that a person of ordinary skill in the art would recognize that modifying peptides by introducing a foreign T -helper cell epitope, like the *Tetanus toxoid* epitope, was an art recognized method of breaking auto-tolerance. The Examiner further argues that it would have been obvious, conventional and within the skill of a person of ordinary skill in the art to identify the exact position for substitution of the *Tetanus toxoid* epitope in the myostatin peptide in order to facilitate the breaking of auto-tolerance. I do not agree.

We had to overcome many obstacles in order to produce the desired immunogenic modified GDF-8 constructs described in the application. In particular, we had to ensure that the inserted foreign T-helper cell epitopes into the GDF-8 polypeptide would be properly presented and recognized as "foreign" by the animal's immune system. And, at the same time, we also had to ensure the modifications would maintain the overall secondary, tertiary and quaternary structure of the GDF-8 polypeptide and that a substantial portion of the original B-lymphocyte epitopes in the native GDF-8 polypeptide was maintained.

I have reviewed the Barker reference and the passages cited by the Examiner. Barker merely states that immunological carriers (e.g. tetanus) can be associated with a myostatin

immunogen of interest. It is clear that the myostatin immunogen is linked to the specified carrier molecule (see col. 9, lines 22-39). The tetanus molecule is not inserted into the myostatin polypeptide itself and the Barker reference is silent with respect to where and how a tetanus toxoid epitope could be inserted into the myostatin peptide.

I have also considered the Examiner's arguments with respect to the knowledge in the art. I agree that it is known in the art that it is possible to modify a self-protein to render it immunogenic. I also agree, and the Specification also states, that foreign- T helper epitopes have been used to modify peptide self-antigens to destroy autotolerance. However, this was not the case with the myostatin peptide. Although the prior art knowledge disclosed the general principles used to render peptides immunogenic, this merely serves as an invitation for the skilled artisan to discover and identify suitable positions to modify in the GDF-8 peptide. It was not known in the art exactly where modifications could be made in the myostatin peptide to break auto-tolerance in an animal while maintaining the overall structure of the polypeptide and preserving a substantial portion of the native B cell epitopes. The present inventors were the first to discover where foreign T-helper epitopes could be introduced in the myostatin peptide through a series of complex sequence and structure analyses conducted between spring 1999 and summer 2000. As a result of these experiments, we determined that certain areas of the native GDF-8 peptide were particularly suited for modification namely, residues 18-41, 49-69 or 79-104 in SEQ ID NO: 11 or 12.

A person of ordinary skill in the art could not immediately identify which portions of the GDF-8 polypeptide could be modified without testing hundreds, if not, thousands of variants. In my opinion, the presently claimed positions for modifications of the GDF-8 peptide could not, in any way, be deduced from the prior art, the statement on page 16, lines 24-30 of the Specification or from the Barker reference. I, therefore, believe that the present invention is both novel and non-obvious over the prior art.

The undersigned hereby declares that all statements made herein are based upon knowledge are true, and that all statements based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: 19/3-2004

Steen Klysner

Steen Klysner

Curriculum Vitae

Name Steen S. Klysner
Born 1961 in Copenhagen, Denmark

Higher education

1984 Bachelor degree in Biochemistry from The University of Copenhagen.

1986 Bachelor degree in sports from The University of Copenhagen

1990 Masters degree (Cand Scient) in Biochemistry from The University of Copenhagen
Thesis: "Purification and characterisation of Group V allergens from grass pollen"

1994 Ph.D. from the Technical University of Denmark (DTU) & Industrial Research Scientist ("Erhvervsforsker") from the Danish "Academy of Technical Sciences" (ATV). Thesis: "Isoallergens: Characterisation of natural and recombinant pollen allergen variants".

Major past and current appointments

Scientist at ALK A/S	1990-1995*
Scientist at Novo Nordisk A/S	1995-1997
Scientist & Project Manager at Pharmexa (the former M&E Biotech) Appointed Senior Scientist in 2002 Appointed Head of Protein Technology in 2003	1997-????

* Ph.D student / Industrial research candidate during employment at ALK, in connection to ATV & DTU from 1992-1994, see "Education".

Experience and qualifications

Main areas of scientific experience lies within protein chemistry (protein production, purification and characterisation) including structure & bioinformatics combined with immunology.

Laboratory experience.

- Preparation of protein extracts from natural sources (e.g. allergen extracts from pollen)
- Expression of recombinant proteins in E.coli, and insect cells (S2-cells)
- Purification of proteins from above sources and yeast, fungi and mammalian cells, using semi to fully automated chromatography systems using various matrices.
- Electrophoresis in general combined with a wide range of visualisation techniques and digital evaluation.
- Specialised in IPG 2D-electrophoresis, including tailoring of systems, immune detection, and methods for validation and evaluation.

- Immunochemical characterisation, including most generally applied immunochemical methods like ELISA, RIE, CIE, CRIE etc.
- General spectroscopy, static laser light scattering (LALLS), Circular Dichroism (CD), amino acid analysis, N-terminal sequencing and MALDI-TOF MS.
- Hands on experience with protein crystal growth, data collection and structure generation from Washington University Medical School's biochemistry department (USA) in the X-ray crystallography group working as Ph.D. student (1993).

Main responsible for *in silico* analyses of protein structures, modelling and variant design at Pharmexa in the past 5 years. Furthermore, responsible for establishing and implementing a bioinformatics platform.

Management
Managing various vaccine projects as project manager at Pharmexa, also involving external collaborators over a period of 5 years.

Presently managing external scientific collaborations as well as Heading the Protein Technology group with overall responsibility for the maintenance and development of the protein chemistry area at Pharmexa.

Member of the Project Evaluation Board as well as a member of the Board of Directors at Pharmexa.

Other experience

- Experience with technology transfer, development work as well as the pre-clinical to clinical process.
- Experienced in teaching and presenting scientific as well as non-scientific issues at all levels.
- Experience with IP issues, including authorship of several patents (listed under publications), strategy and surveillance.
- Involved in inter-company research/development strategy and implementation during employment at ALK.
- Experience with committee work and scientific strategic tasks (e.g. in the fields of "Allergen standardisation " and "In vitro IgE assays" in the US and Europe)
- Scientific referee for the journal "Allergy" in the period '91-'95.

Publications

1990
Matthiesen F, Klysner S and Løwenstein H.: Characteristics of grass pollen allergens. In: Sehon A, Kraft D and Kunkel G (eds). epitopes of atopic allergens. Brussels UCB Institute of Allergy, 1999; 9-13.

1992
Klysner S, Welinder K, Løwenstein H and Matthiesen F.: Group V allergens in grass pollens IV, Similarities in amino acid composition and NH2-terminal sequence of Group V allergens from Lolium perenne, Poa pratensis and Dactylis glomerata. Clin Exp Allergy, 1992; 22; 491-497.

1993
Ipsen H, Klysner S, Larsen JN, Løwenstein H, Matthiesen F, Schou C and Sparholt SH: Allergenic extracts. In Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW and Busse WW (eds): Allergy: principles and practice, edition 4, Chapter 20, Mosby-Year Book, St. Louis 1993; 529-553.

Ipsen H, Schou C and Klysner S.: Epitope mapping of Bet v I and Car b I by monoclonal antibodies. Molecular Biology and Immunology of Allergens, CRC Press Inc. 1993 - Proceedings.

1995
Løwenstein, H., Sparholt, S.H., Klysner, S., Ipsen, H., Larsen, J.N.: The signficance of isoallergenic variations in present and future specific immunotherapy. Proceedings - CIA Meeting, Nantucket Island 1994. Int. Arch. Allergy Immunol., 107:285-289, 1995.

1997
Klysner S & Løwenstein H: Allergen extracts and standardization, Chapter 50 in "Allergy and Allegic Diseases", ed Kay A.B., Blackwell Scientific publications, Oxford 1997.

Proceedings from American Chemical Society, San Fransisco, "Characterization of fungal cellulases for fibre modification. M. Schaulein", L. Lange, S. F. Lassen, M. S. Kaupinen, L. N. Anderson, S. Klysner, J. B. Nielsen

2001
Marc Hertz, Surendran Mahalingam, Iben Dalum, Steen Klysner, Joerg Mattes, Anne Neisig, Søren Mouritsen, Paul S. Foster and Anand Gautam: Active Vaccination Against IL-5 Bypasses Immunological Tolerance and Ameliorates Experimental Asthma, The Journal of Immunology, 2001, 167: 3792–33799.

Participation in congresses and meetings with posters or presentations

XIVth congress of EEACI, Berlin, Sept 17-22 1989
Oral presentation:
S.Klysner, F. Matthiesen & H.Løwenstein: "Characterization of Group V allergens in grasses." (Abstract: Allergologic, p 31, B1366E, 12, 1989)

46'th meeting of AAACI., Baltimore, Maryland, USA, March 23-28 1990
Poster presentation :
Klysner S, Matthiesen F & Løwenstein H. "Affinity purification of grass pollen Allergens using monoclonal antibodies" (Abtract: Supl Journ. All. Clin. Imm. s 279, vol 85, no 1 part 2, jan 1990)

XVII Nordiske kongres i Allergologi, Århus , Denmark, May 17-19 1990
Poster presentation
Matthiesen F, Klysner S & Løwenstein H; "Purification and characterization of group V allergens of four grasses"

The XVth Europeea Congres Allergy and Clinical Immunology, Paris, France, May 1992
Poster presentations:
Klysner S & Ipsen H, "Various Bet v I isoallergen subsets visualized by different binding patterns of monoconal antibodies in 2D immunoblotts of Bet v pollen extracts"

Matthiesen F, Nielsen AK, Søgaard TJ, Klysner S & Løwenstein H., "N-terminal sequences of four immunoaffinity purified grass pollen allergens" (Abstracts: Allergy sup s30, 12,47, 1992.)

Scherax allergie symposium, Berlin August 1992
Oral presentation / lecture:
S.Klysner, "Cross reactivity between grass pollen species"

48'th meeting of AAACI Chicago, marts 1993.
Poster :
S.Klysner, H.Ipsen & J.N.Larsen, "The isoallergenic variation of natural and recombinant Bet v I "(Abstract: Journ.All.Clin.Imm. p281, Vol 91, No 1, Part 2, Jan 1993)

19. Tagung der Deutchen Gesellschaft für allergie- und immunitätsforchung, Potsdam, 21-25 April 1993
Oral presentation:
S.Klysner, Preparation, purification and activity of recombinant allergens.

XVIII Nordic Congres of Allergology, Lund, June 1993
Poster:
H.Ipsen & S.Klysner, Isoallergenic variation of the major allergen of birch pollen, Bet v I (Abstract: Allergy, Sup s 92, 12,48,1993)

Annual meeting of EAACI, Rotterdam, Sept 1993
Poster :
Ipsen H, Klysner S & Larsen JN, "Isoallergenic variation of the major allergen of Birch pollen, Bet v I"

50'th annual meeting of AAA&I, Anaheim, California, March 1994
Poster presentations:
S. Klysner & H. Ipsen, "Variations in the binding of IgE from individual patients to the two-dimensional separation pattern of Bet v I, the major allergen from White Birch".
&
Larsen JN, Casals A, From NB, Ipsen H & Klysner S, "Characterization of purified recombinant Bet v I, produced by fed-batch fermentation".
Ipsen H, Klysner S & Larsen JN, "Epitope mapping of Bet v I isoforms by monoclonal antibodies and the isoallergenic variation of Bet v I from individual Betula verrucosa trees

The Annual meeting of EAACI Stockholm, June 1994.
Poster::
Ipsen H, Larsen LN & Klysner S, "The isoallergenic variation of the major allergen of birch pollen, Bet v I".

XVI Europecan Congres of Allergy and Immunology, Madrid, June 1995
Posters:
Ipsen H, Würtzen PA, Larsen JN, Wissenbach M, Klysner S and Aasmuhl-Olsen S, "Isolation and Characterisation of Group 1 allergens from 11 grass species" (abstract in Supplement to Allergy, No 26, vol 50, page 130 (P-0143), 1995)
&
Wissenbach M, Larsen JN, Würtzen PA & Klysner S. "Cloning and expression of Grass pollen allergens" (Abstract in Supplement to Allergy, No 26, vol 50, page 131 (P-0146), 1995)

Oral presentations + Written contribution
Post Graduate Course: In vivo and in vitro testing in allergy: "Allergens" Klysner S, proceeding in Proceedings from XVI European Congress of Allergy and Immunology, pp 579-583, Monduzzi Editore S.p.a., 1995
&
Round table discussion on Allergen Standardization: "Allergens for in vitro testing" Klysner S, " in Proceedings from XVI European Congress of Allergy and Immunology" pp 585-589, Monduzzi Editore S.p.a., 1995

Novo Nordisk Electrophoresis symposium, Hvidøre 1995
Oral presentation: S. Klysner "Application of IPG 2D PAGE for characterisation of the natural isoallergenic variation of a birch tree pollen allergen"

Novo Nordisk Technology Symposium on Carbohydrate Chemistry, Hvidøre 1996
Poster
S. Klysner, T.V.Borchaardt, T.Halkier, C:C:Fuglsang & M.Schulein; Purification of a hybrid cellulase with two CBDs constructed from family 45 and family 5 enduglucanases from Humicola insolens

Special arrangements:

The talk "Bet v I as a model allergen system" (60 min) given at the allergy units at Washington University (St Louis), Saint Louis University (St Louis), St Paul's Hospital allergy unit (Minneapolis), The Mayo Clinic (Rochester) and National Jewish Clinic allergy unit (Denver) March - June 1993